Microtubules, membranes and cytokinesis

Aaron F. Straight and Christine M. Field

Proper division of the cell requires coordination between chromosome segregation by the mitotic spindle and cleavage of the cell by the cytokinetic apparatus. Interactions between the mitotic spindle, the contractile ring and the plasma membrane ensure that the cleavage furrow is properly placed between the segregating chromosomes and that new membrane compartments are formed to produce two daughter cells. The microtubule midzone is able to stimulate the cortex of the cell to ensure proper ingression and completion of the cleavage furrow. Specialized microtubule structures are responsible for directing membrane vesicles to the site of cell cleavage, and vesicle fusion is required for the proper completion of cytokinesis.

Address: Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.
E-mail: Aaron_Straight@hms.harvard.edu

Current Biology 2000, 10:R760–R770

0960-9822/00/$ – see front matter

Introduction

Cytokinesis is the physical process that divides a cell in two after the completion of mitosis. In most eukaryotic cells, cytokinesis is accomplished by the assembly of an actomyosin ring that contracts to divide the cell midway between the poles of the mitotic spindle. Despite differences among organisms in the execution of cytokinesis, the underlying mechanisms are highly conserved. Actin and myosin play important, if not essential, roles in the cleavage of eukaryotic cells, and the mitotic spindle coordinates the segregation of chromosomes with the site of division.

Cytokinesis in animal cells can be divided into four stages. First, the cell chooses the site of division during the process known as cleavage plane specification or cleavage site selection. Second, cleavage furrow assembly is characterized by protein recruitment to the site of cell division. Third, ingression or contraction of the cleavage furrow introduces membrane barriers separating the cytoplasm of the daughter cells. Finally, the cleavage furrow seals the membrane compartments forming two new cells in the process of completion or abscission. How the cell temporally and spatially coordinates changes in the microtubule cytoskeleton, the actin cytoskeleton and the membrane compartments to accomplish cytokinesis is one of the most interesting problems in cell division.

Here we focus on recent developments in understanding cytokinesis that highlight the roles of microtubules and membranes during cleavage furrow contraction and the completion of cytokinesis. We emphasize the interplay between components of the cytoskeleton and cellular membranes and the importance of these interactions for the process of cytokinesis. We discuss the post-anaphase roles of midzone and furrow microtubules during cytokinesis, the function of microtubules in directing membrane insertion at the furrow region, and the requirements for vesicle fusion at the cleavage site.

Roles of microtubules in cytokinesis

Rearrangement of the microtubule cytoskeleton during mitosis controls the segregation of the chromosomes, the placement of the contractile ring and the completion of cell cleavage. In animal cells, microtubule-dependent processes in cytokinesis can be divided temporally into two parts. Microtubules of the bipolar spindle dictate the position of the cleavage plane midway between the two asters. Subsequently, microtubules of the spindle midzone promote ingression of the cleavage furrow and the completion of cytokinesis. To accomplish these tasks, microtubules must

interact with the cell cortex to mark the site for cleavage furrow assembly and ultimately direct the assembly of actin and myosin at the furrow region. Microtubules must also communicate with the cell cortex and/or membrane to stimulate the ingression of the cleavage furrow, and they must assemble the midzone and midbody microtubule structures required for the completion of cytokinesis.

Microtubules determine the cleavage plane

The importance of microtubules in cytokinesis has been most closely studied in the context of cleavage plane specification, where microtubules dictate the site of cleavage furrow assembly (for a more comprehensive discussion of the problem of cleavage plane specification we refer the reader to one of the many recent reviews on the subject [1–7]). The importance of microtubules in positioning the site of cell division was initially demonstrated by chemical perturbation and micromanipulation studies in sand dollar and sea urchin embryos ([8–12], reviewed in [13]). These studies identified the mitotic apparatus as the determinant of the cleavage plane and demonstrated that cleavage furrow formation required only a pair of properly separated asters. Moreover, the work of Hiramoto [10] argued that, once the mitotic apparatus had delineated the cleavage plane, the spindle and asters were no longer required for the completion of cytokinesis.

In contrast to the studies based on manipulation of marine embryos, the effects of micromanipulating spindles in grasshopper neuroblasts showed that moving the spindle could cause the furrow to regress and a new furrow to form at the new site of the repositioned spindle [14]. Experiments in newt epithelial cells and grasshopper neuroblasts, where the membrane and spindle midzone are closely juxtaposed, argued that the midzone of the spindle can also stimulate the formation of a cleavage furrow [15,16]. In tissue culture cells, physical blocks placed between the spindle midzone and the cortex were found to inhibit furrowing if applied before anaphase [17], arguing for a direct stimulus from the midzone to the cortex. Collectively, these studies suggest that either the asters or the spindle midzone is able to specify the site of division.

Midzone microtubule structures are important for furrow ingression and completion

In addition to their function in furrow positioning, microtubules have been shown in recent studies to be important during furrow ingression and completion. The late stages of cytokinesis appear to be dominated by contraction of the actomyosin ring at the cleavage furrow. There is growing evidence, however, of interdependence between microtubules at the midzone and the actin and myosin network at the cortex.

We shall use the term central spindle or midzone to refer to the region between the separated anaphase chromosomes characterized by arrays of bundled microtubules (Figure 1). As the cleavage furrow contracts, the compact microtubule-containing structure known as the midbody is formed (Figure 1). In tissue culture cells, midzone microtubule bundles are required throughout cytokinesis for proper furrow ingression and completion [18]. These midzone microtubule bundles appear to form from the interzonal microtubules of the mitotic apparatus, but they can also polymerize *de novo* in cells that never contained a bipolar spindle [19].

Studies that have been carried out in cultured cells, the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila* highlight the role of the central spindle, the midzone microtubules and the midbody in furrow ingression and the completion of cytokinesis. Successful completion of cytokinesis requires proteins of diverse classes that localize to the central spindle. These proteins belong to four classes: microtubule motor proteins, components of the telophase disc, and two kinds of signaling molecules — kinases and GTPases and their regulators. Furthermore, regulators of actin polymerization at the cortex can also influence the microtubules of the central spindle.

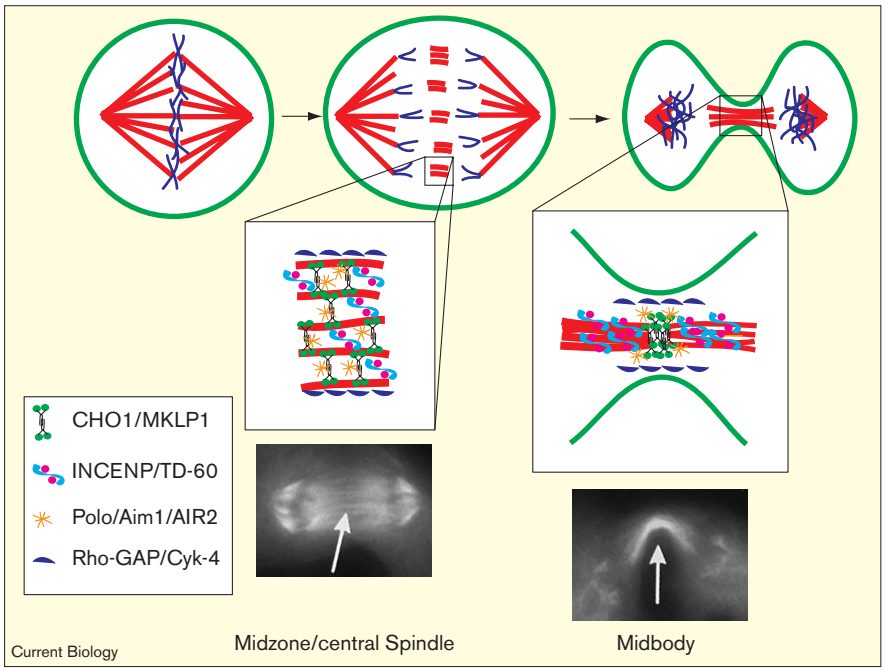
Motor proteins

Kinesins of the CHO1/MKLP1 and KLP3A families are necessary for the formation of the midzone and the completion of cytokinesis. The CHO1/MKLP1 kinesin was identified as a mitotic spindle protein that localized to the spindle midzone and midbody following anaphase and could slide antiparallel microtubules *in vitro* [20–22]. In *Drosophila* mutants for the CHO1/MKLP1 homolog Pavarotti, microtubules failed to bundle in the central spindle and components of the contractile ring failed to assemble, resulting in a cytokinesis failure [23]. The use of RNA mediated interference (RNAi) to inhibit production of the *C. elegans* CHO1/MKLP1 homolog, known as ZEN-4 or CeMKLP1, also resulted in failed cytokinesis. The contractile ring was assembled correctly and furrow ingression was initiated, but midzone microtubule bundles were disrupted and the cleavage furrow regressed [24,25].

The *Drosophila* chromokinesin-related KLP3A protein is also localized to the central spindle and midbody and is required for cytokinesis. *Drosophila* mutants for KLP3A exhibited a loss of the central spindle and no furrow formation, despite a seemingly normal mitosis [26]. The KLP3A mutant flies can, however, mature to adulthood, unlike the *pavarotti* mutants, which die as embryos. These differences may reflect overlapping functions for these kinesins in different stages of development or in different cell types. Alternatively, the differences in phenotype may reflect different functional roles for these kinesins.

Kinesins at the midbody are likely to perform several different roles during cytokinesis. They may be required

Figure 1



Changes in microtubule organization after anaphase. The central spindle and midbody form after segregation of chromosomes at anaphase. Microtubule bundles form in the space between the separated chromosomes. Components of the central spindle begin to assemble in the midzone (left inset). As cytokinesis proceeds, the compact midbody structure is formed containing proteins required for the completion of cytokinesis (right inset). (Adapted from [90].)

to stabilize the microtubule bundles that are necessary for proper furrow ingression and completion, as suggested by the work on the CHO1/MKLP1 class of kinesins. Alternatively, kinesins might be responsible for the microtubule-dependent transport of components essential for the ingression of the furrow and completion of cytokinesis. Other microtubule motor proteins, including the minus-end-directed motor protein dynein, the chromokinesin XKLP1, and the kinetochore-associated kinesin CENP-E reside at the midbody after anaphase, but have not been shown to be directly involved in cytokinesis [27–30].

Chromosomal passenger proteins

The second group of midzone-associated proteins are the telophase disc proteins: the inner centromere protein (INCENP) and the telophase disc antigen (TD-60). These proteins were initially characterized by their unique property of relocalting from chromatin to the central spindle at anaphase [31,32]. Artificially tethering the INCENP protein to the kinetochore, or the production of mutant versions of INCENP, caused a failure in cytokinesis. In tissue culture cells containing mutant INCENP proteins, furrows formed but regressed [33,34]. Examination of the midzone microtubules in INCENP-disrupted cells revealed a lack of microtubule bundling.

Further support for the importance of the midzone microtubule bundles has come from observations on fused cells containing two mitotic spindles. In these cells, cleavage furrows were seen to form, not only between the normal mitotic spindles, but also between spindle poles that were not connected by a spindle or intervening chromatin. In these ectopic furrows, the INCENP protein localized to midzone microtubules [30,34]. Cells that were unable to cleave failed to assemble robust microtubule bundles and lacked INCENP and the kinesin CHO1 in the midzone. Cells that completed cytokinesis formed strong microtubule bundles containing INCENP and CHO1, eventually resulting in the production of a midbody [30]. It is unclear, however, whether the telophase disc proteins are directly involved in microtubule bundling at the midzone. The TD-60 antigen has yet to be cloned and although INCENP associates with microtubules it is not known whether it can function as a microtubule-bundling protein.

Midzone kinases

The first group of signaling molecules important for the assembly and maintenance of the central spindle and midbody are the kinases of the Polo and Aim-1 families. The Polo kinase has been shown to be required for the proper execution of cytokinesis in yeast and *Drosophila* [35,36]. Polo localizes to the spindle midzone at anaphase, and is thought to bind and phosphorylate the kinesin CHO1/MLKP1 [37]. In *polo* mutants, CHO1/MKLP1 localization and central spindle formation were disrupted, and, conversely, *pavarotti* mutants failed to properly recruit Polo kinase to the midzone [23,36]. Another midzone kinase, Aim-1, was identified as a homologue of the Aurora/Ipl1 family of kinases that regulate chromosome

segregation in yeast [38]. Aim-1 behaved as a so-called 'chromosomal passenger' protein and relocalized to the telophase disc at anaphase [39]. Kinase-inactivating mutations in Aim-1 blocked cytokinesis but did not affect chromosome segregation or anaphase spindle elongation [40]. RNAi of the *C. elegans* Aim-1 homolog AIR-2 resulted in normal cleavage furrow formation, as assayed by actin and myosin staining, but the furrows regressed and did not complete cytokinesis [41]. As in the Polo kinase mutants, the CHO1/MKLP1 kinesin did not properly localize to the midzone in AIR-2-deficient cells.

Taken together, these results suggest that the Polo and Aim-1/AIR-2 kinases are important for ensuring the correct assembly of the midzone and midbody microtubule complex and completion of cytokinesis. These kinases may modify substrates, such as CHO1/MKLP1 or other midzone proteins, to promote the assembly of the midzone through microtubule crosslinking or transport of factors required to stabilize the midzone. Alternatively, they may have other roles in signaling changes in the actomyosin network near the midzone or controlling membrane dynamics to ensure the completion phase of cytokinesis.

GTPases

The second group of signaling molecules implicated in cytokinesis are the small GTPases of the Rho, Rac and Cdc42 families. Regulators of GTPase activity, as well as the GTPases themselves, have been found to localize to the contractile ring, direct actin polymerization at the cortex and mediate signaling between the cytoskeleton and the membrane (reviewed in [42]). Recently, it has been shown that a GTPases-activating protein (GAP) involved in cytokinesis is also critical for the structure of midzone microtubules and itself resides at the midbody [43]. The temperature-sensitive *cyk4* mutation in *C. elegans* causes furrow regression and a failure in central spindle formation. Though the *cyk4* gene encodes a promiscuous GAP, it is thought that Cyk4 may act as a cytokinesis-specific GAP for RhoA, as the elimination of only RhoA by RNAi caused a significant cytokinesis defect [43].

Rho GTPases translocate to the cleavage furrow in many organisms, and are thought to regulate actin filament organization [44–46]. Possible downstream effectors of Rho are formin homology (FH) domain-containing proteins, such as *C. elegans* Cyk1. The phenotype of a *cyk1* mutant is very similar to that caused by Rho inhibition: furrow ingression occurs but there is a failure in completion of cytokinesis [47]. Formins are thought to bind the actin regulator profilin through their FH1 domains [48,49], and disruption of formins has been found to cause cytokinesis defects in cultured cells, budding yeast, fission yeast and *Drosophila* [50–53]. It is tempting to speculate that GAP activity at the midzone can activate GTPases in the advancing furrow to stimulate ingression and ensure completion of cytokinesis.

Coordinating events at the midzone and cleavage furrow

Three recent papers [54–56] explore the functional roles of the telophase disc proteins, the kinases and the microtubule motor proteins of the central spindle, and allow us to develop an integrated model of events at the midzone. One important aspect of these studies is the link they have established between the two midzone components INCENP and the aurora kinase Aim-1/AIR-2. In two of the studies [54,55], immunoprecipitates of the INCENP protein from *Xenopus laevis* egg extracts or from HeLa cell extracts were found to contain the kinase Aim-1/AIR-2. Furthermore, recombinant INCENP and Aim-1 were able to associate *in vitro*, demonstrating a direct interaction between these two midzone components [54,55].

Closer inspection of the INCENP protein revealed that it is homologous to the yeast protein Sli15. Sli15 was originally identified genetically as a suppressor of a mutation in Ipl1, the Aim-1/aurora kinase homolog in budding yeast; furthermore, like INCENP and Aim-1, Sli15 and Ipl1 had earlier been found to bind one another both *in vivo* and *in vitro* [57]. RNAi of INCENP in *C. elegans* caused regression of the cleavage furrow and defects in chromosome segregation [55] that were indistinguishable from phenotypes of AIR-2-depleted embryos [55]. The failure of chromosome segregation in the INCENP-depleted embryos is reminiscent of segregation defects caused by expression of mutant forms of INCENP in tissue culture cells: in both cultured cells containing mutant INCENP [54], and *C. elegans* embryos depleted of INCENP [55], Aim-1/AIR-2 fails to properly localize to the midzone and cytokinesis fails.

Two additional papers [58,59] suggest that the BIR-1 protein of *C. elegans*, and its vertebrate homolog survivin, are involved in regulating the structure and assembly of the microtubule midzone. The phenotype of *BIR-1* inhibition was indistinguishable from that of *AIR-2* inhibition, and *BIR-1* inhibition also disrupted localization of AIR-2 protein to the chromosomes and midzone [58]. Like INCENP and Aim-1/AIR-2, both survivin and BIR-1 localize to chromatin prior to anaphase, and to the midzone after anaphase, and *survivin* null mutant mice are phenotypically similar to mice lacking INCENP [59].

The relationship between the INCENP–Aim-1/AIR-2 complex and the ZEN-4/CHO1/MKLP1 microtubule motor protein was investigated through mutant studies in *C. elegans* [56]. In temperature-sensitive mutants for either ZEN-4 or AIR-2, furrow ingression failed to initiate at the non-permissive temperature but cytokinesis was not completed and the furrow regressed. In the *zen-4* mutant, ZEN-4 failed to associate with the midzone and no central spindle formed. In the temperature-sensitive *air-2* mutant, at the non-permissive temperature the cleavage

furrow regressed and the central spindle was disorganized, very similar to the effect of AIR-2 depletion by RNAi. Elegant temperature-shift experiments showed that AIR-2 function was required at metaphase or early anaphase, and was subsequently dispensable. In contrast, ZEN-4 activity was not required until after anaphase, and was necessary through interphase to ensure abscission. In embryos depleted for AIR-2 by temperature shift or RNAi, ZEN-4 does not properly localize to the midzone [41,56]. AIR-2 is thus required early in mitosis to ensure proper placement of ZEN-4.

The observations on INCENP, Aim-1/AIR-2 and ZEN-4 lead to a model for the way chromosome segregation is coordinated with assembly of the midzone and completion of cytokinesis. The INCENP–Aim-1/AIR-2 complex is clearly required for chromosome segregation. One likely model is that INCENP targets Aim-1/AIR-2 to the chromosome, perhaps through its demonstrated interaction with heterochromatin protein 1 [60]. The transition into anaphase might release the INCENP–Aim-1/AIR-2 complex from chromatin, whereupon it can associate with the midzone of the spindle. At the spindle midzone, Aim-1/AIR-2 then acts to ensure the correct localization of ZEN-4 to the midzone microtubule bundles, which is required for cytokinesis to proceed to completion. The *zen-4*, *air-2* double mutant is still able to undergo furrow ingression, but double mutants combining either *zen-4* or *air-2* with the *cyk1* mutation mentioned above completely fail to undergo furrow contraction [56].

Robust ingression of the furrow thus requires both proteins at the spindle midzone and proteins of the cell cortex. One possibility is that the primary role of the INCENP–Aim-1/AIR-2–ZEN4 pathway in cytokinesis is to ensure proper assembly of the central spindle and midzone, so that the furrow can proceed to completion. Coupling the process of chromosome segregation to the process of midzone formation guarantees that cytokinesis is coordinated with chromosome segregation.

The idea that there is an interaction between the central spindle and the cortex is emerging from studies with *Drosophila* (reviewed in [61]). Mutations in *Drosophila* that affect the central spindle appear to have a dramatic influence on the contractile ring. As mentioned above, mutations that affect the central spindle, such as those of KLP3A or *pavarotti*, also disrupt contractile ring formation and cytokinesis [23,26]. Unlike the case with many other eukaryotic cells, it is now evident that in *Drosophila* cells disruption of the contractile ring can also cause a loss of the central spindle. The *Drosophila* profilin mutant *chickadee* is unable to undergo cytokinesis. Examination of spermatids produced by the *chickadee* mutant showed that, not only was the contractile ring unable to form, but the central spindle was absent. The same was true for mutants of the formin homology protein Diaphanous. Disruption of the actin cytoskeleton with cytochalasin D also eliminated the central spindle [62]. These results with *Drosophila* are supported by observations in tissue culture cells, where disruption of filamentous (F) actin with cytochalasin B caused abnormalities in some anaphase and telophase central spindles [63]. These results suggest that, not only does the central spindle affect the structure of the contractile ring, but the contractile ring can influence the structure of the central spindle.

In summary, an extraordinary interplay exists between post-anaphase microtubule arrays and the contraction of the cleavage furrow and the completion of cytokinesis. Midzone microtubule and midbody structures containing structural and signaling elements are assembled after the segregation of chromosomes. These structures affect the location of the division site and progression through cytokinesis. A more detailed understanding of cytokinesis will require better knowledge of the microtubule complexes assembled at the midzone and midbody, the signaling pathways between the spindle midzone and the cell cortex, and the pathways that signal from the actin structures at the membrane back to the microtubules of the spindle.

Microtubules and membranes

The final accomplishment of cytokinesis is to create two new membrane compartments surrounding the cytoplasm and nuclei of the newly formed daughter cells. This requires an interaction between the contractile apparatus and the plasma membrane to constrict the cell. The redistribution of membrane to the site of cleavage is required for cytokinesis. During cytokinesis, membranes must be transported to the cleavage furrow. Once properly relocated to the cleavage furrow, membrane vesicles must fuse with the existing plasma membrane or microvilli must be resorbed to create new surface area for cleavage.

The furrow microtubule array and membrane insertion

During cleavage of the *Xenopus* embryo, new membrane is assembled near the site of the ingressing furrow. The early ultrastructural and particle-marking studies of Bluemink and deLaat [64] on cleaving *Xenopus* embryos suggested that the cleavage furrow membrane does not grow at the expense of the existing embryo membrane, but is newly formed. Radioiodination of cleaving *Xenopus* embryos demonstrated that the furrow is formed by the insertion of new membrane. This new membrane is added to the walls of the furrow near, but not at, the leading edge [65] and is thought to arise from Golgi-derived vesicles that fuse with the ingressing furrow [66].

How are membrane vesicles directed to the site of cleavage furrow ingression? The recent discovery of a microtubule structure called the furrow microtubule array (FMA) required for embryonic cytokinesis highlights a

role for microtubules in membrane deposition [67]. Depolymerization of microtubules after the initiation of embryo cleavage in *Xenopus* blocked the completion of division [68]. Examination of tubulin localization in cleaving *Xenopus* embryos revealed a previously unappreciated parallel array of microtubules at the base of the cleavage furrow (Figure 2). These microtubules appeared to form from midzone microtubule bundles as the furrow contracted. After microtubule depolymerization, however, the FMA microtubules regrew more quickly than astral or spindle microtubules.

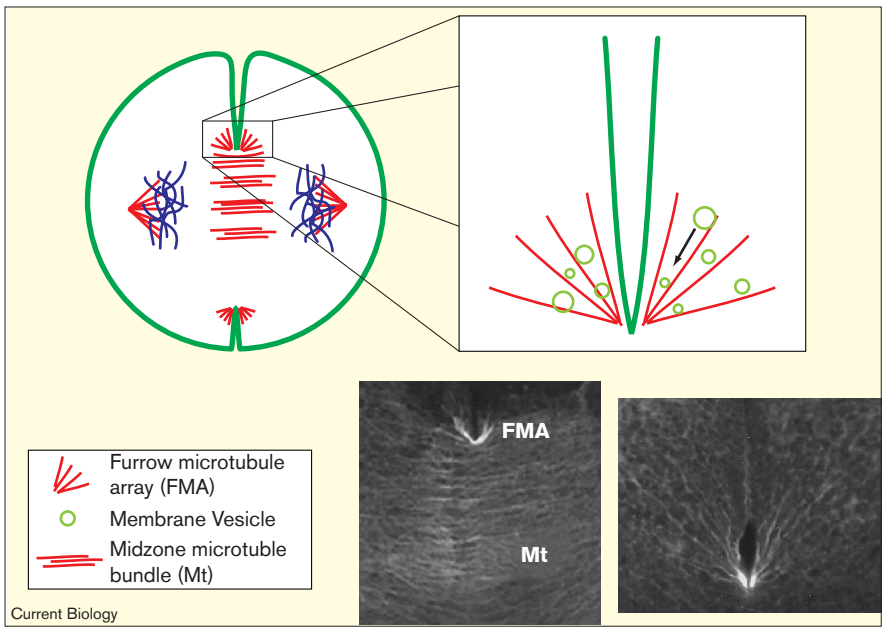
Treatment of embryos with cytochalasin D did not disrupt the localization of FMA microtubules or the insertion of new membrane, but did block furrow ingression [67]. This is reminiscent of the effect of injecting embryos with the Rho GTPase inhibitor, C3 transferase, which resulted in defects in actin assembly and furrowing, but not in the addition of new membrane [46]. Depolymerization of microtubules in the embryo blocked new membrane addition in the furrow and resulted in furrow regression. Cold-shock disruption of the FMA caused rupture of the embryo along the cleavage plane, and this rupture could be blocked by simultaneous treatment with cytochalasin B [67]. These results argue that the FMA is required for membrane addition at the cleavage furrow in the early embryo, and that without addition of new membrane the *Xenopus* embryo is unable to cleave because of insufficient surface area. Furthermore, the FMA has also been observed in the sea urchin, where treatment of embryos with nocodazole or colchicine blocked cleavage at the late stage of abscission [69].

In zebrafish embryos, a prominent FMA associates with the furrow in dividing blastomeres. Treatment of these embryos with nocodazole also blocked the addition of new membrane, as assayed by staining for β-catenin. Furthermore, labeling of membranes in the dividing zebrafish embryo showed that membrane clustering occurs at the site of the furrow, and this was dispersed upon microtubule depolymerization [70]. A zebrafish mutant, *nebel*, has now been identified with defects in both the FMA and the process of membrane addition at the furrow [71]. The *nebel* mutant failed in both ingression and abscission during cytokinesis, a phenotype similar to the effects of microtubule disruption. Staining of *nebel* embryos for tubulin showed a normal mitotic spindle and asters, but a reduced FMA. Membrane insertion in *nebel* embryos was also defective, as assayed by β-catenin or concanavalin A staining.

It seems likely that microtubule-dependent transport of membrane components to the cleavage furrow is important for cleavage. Although the product of the *nebel* gene is not yet known, examination of other aspects of the *nebel* mutant phenotype suggests that the *nebel* gene product is involvement in microtubule-based transport. Zebrafish, like a number of other organisms, contain a specialized region of embryonic cytoplasm called the germ plasm, which is required for proper differentiation of germ cells. Germ plasm organization in *Xenopus* embryos is dependent on microtubules [72]. In either microtubule-disrupted or *nebel* mutant zebrafish embryos the germ plasm and an RNA component of the germ plasm, *vasa* RNA, are both mislocalized. One attractive candidate for the product of the *nebel* gene would be a microtubule-dependent motor protein.

Figure 2

The furrow microtubule array (FMA) directs the insertion of membrane into the cleavage furrow. At the top is shown a schematic illustration of the FMA. At the bottom are images of FMA and midzone microtubules in the *Xenopus* embryo (reproduced from [65]). Microtubules of the FMA are distinct from the midzone microtubule bundles.



The kinesin Xklp1 is known to be important for germ plasm organization and localizes to the midbody during cytokinesis [73]; it is unclear, however, whether Xklp1 is directly involved in cytokinesis in *Xenopus* embryos.

If microtubule-dependent transport is important for the completion of cytokinesis, then the polarity of the microtubules in the midbody and the FMA becomes an important consideration. Immunogold staining of the midbody with antibodies against γ-tubulin suggests that the plus ends of the microtubules are located in the center of the midbody and the minus ends at the periphery [74]. Furthermore, inhibition of γ-tubulin during mitosis results in disorganization of the midbody and a block to the completion of cytokinesis [74,75]. The observation that FMA microtubules can polymerize before the astral microtubules polymerize suggests that there is a microtubule nucleating center near the furrow, but it is unclear whether that center is at the base of the furrow or distal to the furrow. Both plus-end-directed and minus-end-directed microtubule motors localize to the midbody region. Thus it is necessary to determine the polarity of microtubules involved in membrane addition and the completion of cytokinesis, and the nature of the motor proteins that use those microtubules.

How are the FMA microtubules related to the midzone and midbody microtubules in other systems? It is tempting to speculate that the FMA microtubules arose as an adaptation for embryonic systems that require relatively more membrane insertion than somatic cells, where the demands for membrane addition are less. The midzone and midbody microtubules may be sufficient to direct membrane insertion into somatic cell cleavage furrows, whereas in large embryos, the mitotic spindle is often far removed from the site of furrowing, necessitating independent microtubule systems. In agreement with such a model, once the *Xenopus* embryo reaches the mid-blastula transition, where the nuclear to cytoplasmic ratio is closer to that of a somatic cell, the FMA disappears and is replaced with a conventional midbody [67].

Vesicle fusion and cytokinesis

Once vesicles have been transported to the cleavage furrow they must fuse with the plasma membrane at the furrow. These fusion events must be constrained to the cell regions that require the addition of new membrane and must be coordinated with the ingressing furrow. Genetic studies carried out in the plant *Arabidopsis*, the fly *Drosophila* and the worm *C. elegans* have revealed the importance of membrane fusion in cytokinesis.

Plant cells accomplish cytokinesis, not by contracting an actomyosin ring, but rather by assembling membrane at the midline of the cell. Plant cytokinesis begins with the transport of Golgi-derived vesicles to the center of the cell. A structure called the phragmoplast is assembled at the division plane, consisting of a set of interdigitating microtubules and sets of actin filaments. Vesicles are transported toward the plus ends of phragmoplast microtubules at the center of the cell, where they fuse into a membranous network called the cell plate. Eventually the fused vesicles form a membrane barrier between cells, followed by assembly of the new cell wall. The first molecular link between membrane fusion and cytokinesis came from the study of the *Arabidopsis* mutant *knolle*, which has multinucleate cells as a result of failed cell-plate and cross-wall synthesis [76]. This is reminiscent of the failure of cytokinesis caused by microtubule depolymerization [77]. Cloning of the *KNOLLE* gene revealed that it encodes a syntaxin, a member of the t-SNARE family of proteins.

SNAREs are proteins required for vesicle fusion in eukaryotes, and can be divided into two classes: t-SNAREs, which reside on target membranes, and v-SNAREs on vesicle membranes. Specific binding between a t-SNARE and a v-SNARE mediates fusion of the vesicle and target membranes, a process that has been best characterized in the case of ER-to-Golgi and Golgi-to-plasma membrane transport (reviewed in [78,79]). Staining with antibodies against the KNOLLE protein showed that it resides at the midline of the cell during cytokinesis. Furthermore, the dynamin-like protein ADL1, which is transported to the midline by vesicle transport, was found to be localized normally in *knolle* mutant plants. Electron microscopic observation of the *knolle* cells showed vesicle accumulation at the phragmoplast; thus, transport of vesicles along microtubules to the site of cytokinesis was normal in the mutant cells, but the fusion of these vesicles with their target membranes was defective [80]. Recent evidence has shown that the uncloned *Arabidopsis* gene *KEULE* is also involved in vesicle fusion, and that *knolle* and *keule* mutations interact in double mutant plants [81]. Thus, *KEULE* might encode a v-SNRARE or other protein involved in vesicle fusion and the process of cell-plate formation.

Syntaxin-mediated fusion of vesicles to the plasma membrane is also required for cytokinesis in *Drosophila* and *C. elegans*. The *Drosophila* embryo undergoes 13 syncytial nuclear cycles prior to cellularization. During cycle 14, membrane invagination and constriction isolates nuclei, converting the syncytial blastoderm into a multicellular embryo. The process of cellularization requires the insertion of large amounts of new membrane into the plasma membrane to provide surface area for the invaginating cellularization furrows ([82], reviewed in [83]). Syntaxin1 in *Drosophila* appears in the early embryo before the development of the nervous system. Immunostaining with antibodies to *Drosophila* syntaxin1 showed that it localizes to the membrane of the cellularization furrow. Syntaxin1 mutant flies exhibit no obvious phenotype until cycle 13, but they

show a dramatic failure of cellularization in the next cycle. The cellularization front in *syntaxin* mutants completely fails to ingress, suggesting that little or no membrane insertion occurs at the sites of cellularization [84].

The ability to deplete the *C. elegans* embryo of syntaxins by RNAi allowed a direct test of their role in embryonic cytokinesis [85]. The complete genome of *C. elegans* revealed eight genes encoding syntaxin-related proteins, the product of each of which was separately depleted in the developing embryo. Depletion of two of the eight syntaxins resulted in embryonic lethality, but in only one case, the syntaxin1 homolog Syn-4, was depletion found to cause cells to become multinucleate. Time-lapse microscopy of inhibited embryos revealed two major defects. First, nuclear envelope reformation was impaired, such that two nuclei were often formed in a blastomere. Second, cleavage furrows either ingressed but did not complete cytokinesis and regressed, or they failed to ingress. Another defect indicative of a failure to complete cytokinesis was the resorption of the polar body into the embryo. Staining for the Syn-4 protein in the early embryo showed that it localizes to the cleavage furrow and remains associated with the region of the plasma membrane left during furrow ingression [85].

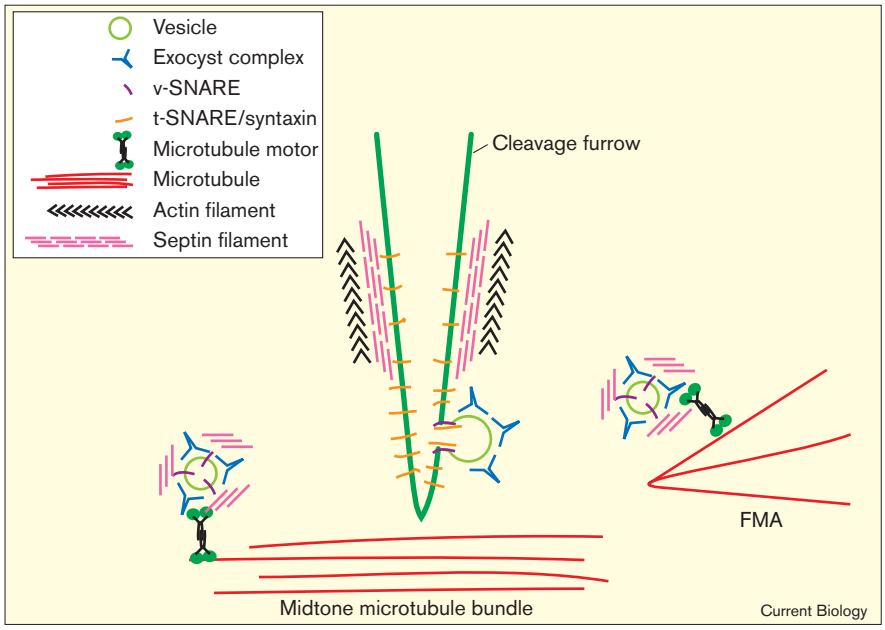
The discovery of a link between syntaxins, exocytosis and the septin complex may be important for understanding cytokinesis. The septins are a highly conserved group of GTP-binding proteins first identified by the cell-cycle defects their mutations cause in budding yeast. Analysis of septins in *Drosophila*, yeast and tissue-culture cells showed that they localize to the contractile ring and that they are required for the completion of cytokinesis. Septins show actin-dependent localization with stress fibers during interphase and with the contractile ring during cytokinesis (reviewed in [86]). The discovery that septins can interact with the Sec6/8 or exocyst complex implicates them in the process of membrane targeting.

The exocyst is a complex of at least seven subunits that is required for proper function of the secretory pathway. It is thought that the exocyst complex targets vesicles to specific sites on the plasma membrane [87]. Immunoprecipitation of the exocyst from rat brain revealed four associated proteins, three of which were identified as septin proteins [88]. A second link in this pathway may be through specific interaction of the septin complex with the syntaxins. Isolation of synaptosomes revealed that the septin CDCrel-1 associates specifically with synaptic vesicles. When tested by co-immunoprecipitation, both CDCrel-1 and the septin Nedd5 were found to associate with these synaptic vesicles, along with the t-SNARE syntaxin1A. The interaction between septins and syntaxin1A appeared, on the basis of *in vitro* binding of the GST-fusion proteins, to be direct. The effects of expressing wild-type and mutant forms of CDCrel-1 in transfected cells indicate that wild-type septins inhibit the process of exocytosis [89].

How do the syntaxins, septins and the exocyst complex regulate cytokinesis? One possible model is that the exocyst complex directs vesicles to the site of membrane fusion at the cleavage furrow (Figure 3). The septins may

Figure 3

A model for regulated transport and fusion of membrane vesicles at the cleavage site. Microtubule motors participate in transport of vesicles to furrow region. Vesicles associated with the exocyst complex and v-SNARES are targeted to plasma membrane of furrow. Septins at furrow region and t-SNARES ensure correct insertion of vesicles into the plasma membrane near the site of furrow invagination.



act during cytokinesis to target membrane insertion to the furrow, or they may link the cytoskeletal elements of the cleavage furrow with the membrane components. Once the vesicles required for the completion of cytokinesis reach the site of constriction, their ultimate fusion with the plasma membrane is controlled by the syntaxins on the plasma membrane (Figure 3). There is little evidence from mutation or inhibition studies for the involvement of the exocyst complex in cytokinesis, although this may be due to the more severe effects of exocyst mutants on the process of secretion.

The exact role of the septin complex in the process of membrane insertion is still unclear. The inhibition of exocytosis by expression of wild-type septins argues for an inhibitory role in the completion of cytokinesis, or at least a regulatory role to confine the area of membrane insertion to the correct location (Figure 3). Furthermore, mutations in v-SNAREs or other fusion proteins that affect cytokinesis have yet to be characterized.

Summary

Dissecting the processes required for the completion of cytokinesis is a challenging endeavor. In addition to their role in positioning the site of division, microtubules are critical for the proper completion of cytokinesis. The construction of the spindle midzone and the midbody microtubule structures requires the coordinated activities of many proteins with diverse functions. Defects in components of the midzone and midbody cause failures in cleavage furrow ingression or the completion of cytokinesis. Microtubule-dependent transport of vesicles to the site of cytokinesis is clearly required in embryonic, plant and somatic systems. Finally, regulated insertion of membrane vesicles at the site of cytokinesis is necessary for both furrow ingression and abscission.

Acknowledgements

We would like to thank Alison Farrell, Julie Canman, Mimi Shirasu and Jennifer Tirnauer for critical reading of the manuscript. A.S. was supported by funds from the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation and C.F. by NIH GM 23928 awarded to T. Mitchison.

References

1. Robinson DN, Spudich JA: **Towards a molecular understanding of cytokinesis.** *Trends Cell Biol* 2000, **10:**228-237.
2. Field C, Li R, Oegema K: **Cytokinesis in eukaryotes: a mechanistic comparison**. *Curr Opin Cell Biol* 1999, **11:**68-80.
3. Glotzer M: **The mechanism and control of cytokinesis.** *Curr Opin Cell Biol* 1997, **9:**815-823.
4. Hales KG, Bi E, Wu JQ, Adam JC, Yu IC, Pringle JR: **Cytokinesis: an emerging unified theory for eukaryotes?** *Curr Opin Cell Biol* 1999, **11:**717-725.
5. Sisson JC, Rothwell WF, Sullivan W: **Cytokinesis: lessons from rappaport and the *Drosophila* blastoderm embryo.** *Cell Biol Int Rep* 1999, **23:**871-876.
6. Smith LG: **Divide and conquer: cytokinesis in plant cells.** *Curr Opin Plant Biol* 1999, **2:**447-453.
7. Wheatley SP: **Updates on the mechanics and regulation of cytokinesis in animal cells.** *Cell Biol Int Rep* 1999, **23:**797-803.
8. Beams HW, Evans TC: **Some effects of colchicine upon the first cleavage in *Arbacia punctulata*.** *Biol Bull* 1940, **79:**188-198.
9. Swann MM, Mitchison JM: **Cleavage of sea-urchin eggs in colchicine.** *J Exp Biol* 1953, **30:**506-514.
10. Hiramoto Y: **Cell division without mitotic apparatus in sea urchin eggs.** *Exp Cell Res* 1956, **11:**630-636.
11. Hiramoto Y: **Further studies on cell division without mitotic apparatus in sea urchin eggs.** *J Cell Biol* 1965, **25:**161-166.
12. Rappaport R: **Experiments concerning the cleavage stimulus in sand dollar eggs.** *J Exp Zool* 1961, **144:**225-231.
13. Rappaport R: *Cytokinesis in Animal Cells*. Cambridge University Press: Cambridge; 1996.
14. Kawamura K: **Studies on cytokinesis in neuroblasts of the grasshopper, *Chortophaga viridifasciata* (De Geer).** *Exp Cell Res* 1960, **21:**1-18.
15. Kawamura K: **Microdissection studies on the dividing neuroblast of the grasshopper, with special reference to the mechanism of unequal cytokinesis.** *Exp Cell Res* 1977, **106:**127-137.
16. Rappaport R, Rappaport BN: **Establishment of cleavage furrows by the mitotic spindle.** *J Exp Zool* 1974, **189:**189-196.
17. Cao LG, Wang YL: **Signals from the spindle midzone are required for the stimulation of cytokinesis in cultured epithelial cells.** *Mol Biol Cell* 1996, **7:**225-232.
18. Wheatley SP, Wang Y: **Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells.** *J Cell Biol* 1996, **135:**981-989.
19. Canman JC, Hoffman DB, Salmon ED: **The role of pre- and post-anaphase microtubules in the cytokinesis phase of the cell cycle.** *Curr Biol* 2000, **10:**611-614.
20. Sellitto C, Kuriyama R: **Distribution of pericentriolar material in multipolar spindles induced by colcemid treatment in Chinese hamster ovary cells.** *J Cell Sci* 1988, **89:**57-65.
21. Nislow C, Sellitto C, Kuriyama R, McIntosh JR: **A monoclonal antibody to a mitotic microtubule-associated protein blocks mitotic progression.** *J Cell Biol* 1990, **111:**511-522.
22. Nislow C, Lombillo VA, Kuriyama R, McIntosh JR: **A plus-end-directed motor enzyme that moves antiparallel microtubules *in vitro* localizes to the interzone of mitotic spindles.** *Nature* 1992, **359:**543-547.
23. Adams RR, Tavares AA, Salzberg A, Bellen HJ, Glover DM: **Pavarotti encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis.** *Genes Dev* 1998, **12:**1483-1494.
24. Raich WB, Moran AN, Rothman JH, Hardin J: **Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4.** *Mol Biol Cell* 1998, **9:**2037-2049.
25. Powers J, Bossinger O, Rose D, Strome S, Saxton W: **A nematode kinesin required for cleavage furrow advancement.** *Curr Biol* 1998, **8:**1133-1136.
26. Williams BC, Riedy MF, Williams EV, Gatti M, Goldberg ML: **The *Drosophila* kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis.** *J Cell Biol* 1995, **129:**709-723.
27. Karki S, LaMonte B, Holzbaur EL: **Characterization of the p22 subunit of dynactin reveals the localization of cytoplasmic dynein and dynactin to the midbody of dividing cells.** *J Cell Biol* 1998, **142:**1023-1034.
28. Vernos I, Raats J, Hirano T, Heasman J, Karsenti E, Wylie C: **Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning.** *Cell* 1995, **81:**117-127.
29. Yen TJ, Li G, Schaar BT, Szilak I, Cleveland DW: **CENP-E is a putative kinetochore motor that accumulates just before mitosis.** *Nature* 1992, **359:**536-539.
30. Savoian MS, Earnshaw WC, Khodjakov A, Rieder CL: **Cleavage furrows formed between centrosomes lacking an intervening spindle and chromosomes contain microtubule bundles, INCENP, and CHO1 but not CENP-E.** *Mol Biol Cell* 1999, **10:**297-311.
31. Andreassen PR, Palmer DK, Wener MH, Margolis RL: **Telophase disc: a new mammalian mitotic organelle that bisects telophase cells with a possible function in cytokinesis.** *J Cell Sci* 1991, **99:**523-534.
32. Cooke CA, Heck MM, Earnshaw WC: **The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis.** *J Cell Biol* 1987, **105:**2053-2067.
33. Mackay AM, Ainsztein AM, Eckley DM, Earnshaw WC: **A dominant mutant of inner centromere protein (INCENP), a chromosomal protein, disrupts prometaphase congression and cytokinesis.** *J Cell Biol* 1998, **140:**991-1002.

34. Eckley DM, Ainsztein AM, Mackay AM, Goldberg IG, Earnshaw WC: **Chromosomal proteins and cytokinesis: patterns of cleavage furrow formation and inner centromere protein positioning in mitotic heterokaryons and mid-anaphase cells.** *J Cell Biol* 1997, **136:**1169-1183.
35. Ohkura H, Hagan IM, Glover DM: **The conserved *Schizosaccharomyces pombe* kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells.** *Genes Dev* 1995, **9:**1059-1073.
36. Carmena M, Riparbelli MG, Minestrini G, Tavares AM, Adams R, Callaini G, Glover DM: ***Drosophila* polo kinase is required for cytokinesis.** *J Cell Biol* 1998, **143:**659-671.
37. Lee KS, Yuan YL, Kuriyama R, Erikson RL: **Plk is an M-phase-specific protein kinase and interacts with a kinesin- like protein, CHO1/MKLP-1.** *Mol Cell Biol* 1995, **15:**7143-7151.
38. Chan CS, Botstein D: **Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast.** *Genetics* 1993, **135:**677-691.
39. Martineau-Thuillier S, Andreassen PR, Margolis RL: **Colocalization of TD-60 and INCENP throughout G2 and mitosis: evidence for their possible interaction in signalling cytokinesis.** *Chromosoma* 1998, **107:**461-470.
40. Terada Y, Tatsuka M, Suzuki F, Yasuda Y, Fujita S, Otsu M: **AIM-1: a mammalian midbody-associated protein required for cytokinesis.** *EMBO J* 1998, **17:**667-676.
41. Schumacher JM, Golden A, Donovan PJ: **AIR-2: An Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in Caenorhabditis elegans embryos.** *J Cell Biol* 1998, **143:**1635-1646.
42. Prokopenko SN, Saint R, Bellen HJ: **Untying the Gordian knot of cytokinesis. Role of small G proteins and their regulators.** *J Cell Biol* 2000, **148:**843-848.
43. Jantsch-Plunger V, Gonczy P, Romano A, Schnabel H, Hamill D, Schnabel R, Hyman AA, Glotzer M: **CYK-4. A rho family gtpase activating protein (gap) required for central spindle formation and cytokinesis.** *J Cell Biol* 2000, **149:**1391-1404.
44. Takaishi K, Sasaki T, Kameyama T, Tsukita S, Takai Y: **Translocation of activated Rho from the cytoplasm to membrane ruffling area, cell-cell adhesion sites and cleavage furrows.** *Oncogene* 1995, **11:**39-48.
45. Nishimura Y, Nakano K, Mabuchi I: **Localization of Rho GTPase in sea urchin eggs.** *FEBS Lett* 1998, **441:**121-126.
46. Drechsel DN, Hyman AA, Hall A, Glotzer M: **A requirement for Rho and Cdc42 during cytokinesis in *Xenopus* embryos.** *Curr Biol* 1997, **7:**12-23.
47. Swan KA, Severson AF, Carter JC, Martin PR, Schnabel H, Schnabel R, Bowerman B: **cyk-1: a *C. elegans* FH gene required for a late step in embryonic cytokinesis.** *J Cell Sci* 1998, **111:**2017-2027.
48. Frazier JA, Field CM: **Actin cytoskeleton: are FH proteins local organizers?** *Curr Biol* 1997, **7:**R414-417.
49. Wasserman S: **FH proteins as cytoskeletal organizers.** *Trends Cell Biol* 1998, **8:**111-115.
50. Chang F, Woollard A, Nurse P: **Isolation and characterization of fission yeast mutants defective in the assembly and placement of the contractile actin ring.** *J Cell Sci* 1996, **109:**131-142.
51. Castrillon DH, Wasserman SA: **Diaphanous is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the limb deformity gene.** *Development* 1994, **120:**3367-3377.
52. Kohno H, Tanaka K, Mino A, Umikawa M, Imamura H, Fujiwara T, Fujita Y, Hotta K, Qadota H, Watanabe T, *et al.*: **Bni1p implicated in cytoskeletal control is a putative target of Rho1p small GTP binding protein in *Saccharomyces cerevisiae*.** *EMBO J* 1996, **15:**6060-6068.
53. Watanabe N, Madaule P, Reid T, Ishizaki T, Watanabe G, Kakizuka A, Saito Y, Nakao K, Jockusch BM, Narumiya S: **p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin.** *EMBO J* 1997, **16:**3044-3056.
54. Adams RR, Wheatley SP, Gouldsworthy AM, Kandels-Lewis SE, Carmena M, Smythe C, Gerloff DL, Earnshaw WC: **INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow.** *Curr Biol* 2000, **10:**1075-1078.
55. Kaitna S, Mendoza M, Jantsch-Plunger V, Glotzer M: **Incenp and an Aurora-like kinase form a complex that is essential for chromosome segregation and for efficient completion of cytokinesis.** *Curr Biol* 2000, **10:**1172-1181.
56. Severson AF, Hamill DR, Carter JC, Bowerman B: **The Aurora/Ipl1p-related kinase AIR-2 functions at metaphase to recruit the kinesin-like protein ZEN-4 to the mitotic spindle midzone and is required for cytokinesis.** *Curr Biol* 2000 **10:**1162-1171.
57. Kim JH, Kang JS, Chan CS: **Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerevisiae*.** *J Cell Biol* 1999, **145:**1381-1394.
58. Speliotes EK, Uren A, Vaux D, Horvitz HR: **The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone.** *Mol Cell* 2000, **6:**211-223.
59. Uren AG, Wong L, Pakusch M, Fowler KJ, Burrows FJ, Vaux DL, Choo KHA: **Localization and deletion phenotype of survivin resembles those of the centromere protein Incenp.** *Curr Biol*, in press.
60. Ainsztein AM, Kandels-Lewis SE, Mackay AM, Earnshaw WC: **INCENP centromere and spindle targeting: identification of essential conserved motifs and involvement of heterochromatin protein HP1.** *J Cell Biol* 1998, **143:**1763-1774.
61. Gatti M, Giansanti MG, Bonaccorsi S: **Relationships between the central spindle and the contractile ring during cytokinesis in animal cells.** *Microsc Res Tech* 2000, **49:**202-208.
62. Giansanti MG, Bonaccorsi S, Williams B, Williams EV, Santolamazza C, Goldberg ML, Gatti M: **Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis.** *Genes Dev* 1998, **12:**396-410.
63. Cimini D, Fioravanti D, Tanzarella C, Degrassi F: **Simultaneous inhibition of contractile ring and central spindle formation in mammalian cells treated with cytochalasin B.** *Chromosoma* 1998, **107:**479-485.
64. Bluemink JG, Laat SWd: **New membrane formation during cytokinesis in normal and cytochalasin B-treated eggs of *Xenopus laevis*. I. Electron microscope observations.** *J Cell Biol* 1973, **59:**89-108.
65. Byers TJ, Armstrong PB: **Membrane protein redistribution during *Xenopus* first cleavage.** *J Cell Biol* 1986, **102:**2176-2184.
66. Singal PK, Sanders EJ: **An ultrastructural study of the first cleavage of *Xenopus* embryos.** *J Ultrastruct Res* 1974, **47:**433-451.
67. Danilchik MV, Funk WC, Brown EE, Larkin K: **Requirement for microtubules in new membrane formation during cytokinesis of *Xenopus* embryos.** *Dev Biol* 1998, **194:**47-60.
68. Sawai T, Yomota A: **Cleavage plane determination in amphibian eggs.** *Ann New York Acad Sci* 1990, **582:**40-49.
69. Larkin K, Danilchik MV: **Microtubules are required for completion of cytokinesis in sea urchin eggs.** *Dev Biol* 1999, **214:**215-226.
70. Jesuthasan S: **Furrow-associated microtubule arrays are required for the cohesion of zebrafish blastomeres following cytokinesis.** *J Cell Sci* 1998, **111:**3695-3703.
71. Pelegri F, Knaut H, Maischein HM, Schulte-Merker S, Nüsslein-Volhard C: **A mutation in the zebrafish maternal-effect gene *nebel* affects furrow formation and vasa RNA localization.** *Curr Biol* 1999, **9:**1431-1440.
72. Ressom RE, Dixon KE: **Relocation and reorganization of germ plasm in *Xenopus* embryos after fertilization.** *Development* 1988, **103:**507-518.
73. Robb DL, Heasman J, Raats J, Wylie C: **A kinesin-like protein is required for germ plasm aggregation in *Xenopus*.** *Cell* 1996, **87:**823-831.
74. Shu HB, Li Z, Palacios MJ, Li Q, Joshi HC: **A transient association of gamma-tubulin at the midbody is required for the completion of cytokinesis during the mammalian cell division.** *J Cell Sci* 1995, **108:**2955-2962.
75. Julian M, Tollon Y, Lajoie-Mazenc I, Moisand A, Mazarguil H, Puget A, Wright M: **gamma-Tubulin participates in the formation of the midbody during cytokinesis in mammalian cells.** *J Cell Sci* 1993, **105:**145-156.
76. Lukowitz W, Mayer U, Jurgens G: **Cytokinesis in the Arabidopsis embryo involves the syntaxin-related KNOLLE gene product.** *Cell* 1996, **84:**61-71.
77. Mcintosh K, Pickett-Heaps JD, Gunning BES: **Cytokinesis in *Spirogyra*: integration of cleavage and cell-plate formation.** *Int J Plant Sci* 1995, **156:**1-8.
78. Gerst JE: **SNAREs and SNARE regulators in membrane fusion and exocytosis.** *Cell Mol Life Sci* 1999, **55:**707-734.
79. Hay JC, Scheller RH: **SNAREs and NSF in targeted membrane fusion.** *Curr Opin Cell Biol* 1997, **9:**505-512.

80. Lauber MH, Waizenegger I, Steinmann T, Schwarz H, Mayer U, Hwang I, Lukowitz W, Jurgens G: **The Arabidopsis KNOLLE protein is a cytokinesis-specific syntaxin.** *J Cell Biol* 1997, **139:**1485-1493.
81. Waizenegger I, Lukowitz W, Assaad F, Schwarz H, Jürgens G, Mayer U: **The *Arabidopsis KNOLLE* and *KEULE* genes interact to promote vesicle fusion during cytokinesis.** *Curr Biol*, in press.
82. Loncar D, Singer SJ: **Cell membrane formation during the cellularization of the syncytial blastoderm of *Drosophila*.** *Proc Natl Acad Sci USA* 1995, **92:**2199-2203.
83. Warn RM, Warn A, Planques V, Robert-Nicoud M: **Cytokinesis in the early *Drosophila* embryo.** *Ann New York Acad Sci* 1990, **582:**222-232.
84. Burgess RW, Deitcher DL, Schwarz TL: **The synaptic protein syntaxin1 is required for cellularization of *Drosophila* embryos.** *J Cell Biol* 1997, **138:**861-875.
85. Jantsch-Plunger V, Glotzer M: **Depletion of syntaxins in the early Caenorhabditis elegans embryo reveals a role for membrane fusion events in cytokinesis.** *Curr Biol* 1999, **9:**738-745.
86. Field CM, Kellogg D: **Septins: cytoskeletal polymers or signalling GTPases?** *Trends Cell Biol* 1999, **9:**387-394.
87. Hsu SC, Hazuka CD, Foletti DL, Scheller RH: **Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex.** *Trends Cell Biol* 1999, **9:**150-153.
88. Hsu SC, Hazuka CD, Roth R, Foletti DL, Heuser J, Scheller RH: **Subunit composition, protein interactions, and structures of the mammalian brain sec6/8 complex and septin filaments.** *Neuron* 1998, **20:**1111-1122.
89. Beites CL, Xie H, Bowser R, Trimble WS: **The septin CDCrel-1 binds syntaxin and inhibits exocytosis.** *Nat Neurosci* 1999, **2:**434-439.
90. Wheatley SP, Hinchcliffe EH, Glotzer M, Hyman AA, Sluder G, Wang Y: **CDK1 inactivation regulates anaphase spindle dynamics and cytokinesis *in vivo*.** *J Cell Biol* 1997, **138:**385-393.